National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1563

Cholesterol and Fat-Soluble Vitamins in Coconut Oil (Natural and Fortified)

This Standard Reference Material (SRM) is intended primarily for use in the development and validation of analytical methods for the determination of cholesterol, fat-soluble vitamins, fat, and fatty acids in a lipid matrix. SRM 1563 consists of ten ampoules per unit: five ampoules of natural coconut oil (1563-1) and five ampoules of fortified coconut oil (1563-2). Each ampoule contains approximately 4 mL of oil.

Certified Concentration Values in SRM 1563-2: The certified values are expressed as mass fractions in units of mg/kg and also as concentrations in units of mg/L at 23 °C for user convenience [1]. The certified values for the levels of cholesterol, vitamin E as *dl*-α-tocopheryl acetate, and vitamin D_2 as ergocalciferol in the fortified coconut oil are shown in Table 1. These values are based on combined results obtained from (a) gravimetry and isotope dilution/gas chromatography/mass spectrometry (ID/GC/MS) for determination of total cholesterol and (b) gravimetry and liquid chromatography (LC) for determination of ergocalciferol and *dl*-α-tocopheryl acetate.

Reference Concentration Values in SRM 1563-1 and 1563-2: Reference concentration values for fat and fatty acids are provided in Table 2. The reference values in this table were derived from results reported by four collaborating laboratories in an interlaboratory comparison exercise. Reference values are noncertified values that are the best estimates of the true values; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Information Concentration Values in SRM 1563-1 and 1563-2: Information values for cholesterol, retinyl acetate, ergocalciferol, and *dl*-α-tocopheryl acetate in the natural oil (1563-1) and retinyl acetate in the fortified oil (1563-2) are provided in Table 3. For the natural oil, the mass fractions/concentrations are provided as information values because of the low levels of the constituents. For the fortified oil, the value for retinyl acetate has changed since the original certification. To date, data are not available to determine the rate of degradation. Therefore, uncertainties are not assigned, and the values are provided for information only. Information concentration values for additional fatty acids are provided in Table 4. The information values in this table were derived from results reported by four collaborating laboratories. These are noncertified values with no reported uncertainties as there is insufficient information to assess uncertainties. The information values are given to provide additional characterization of the material.

Expiration of Certification: The certification of this SRM lot is valid until **31 December 2001**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see Instructions for Use). However, the certification is nullified if the SRM is damaged, contaminated, or modified.

The support aspects involved in the revision and issuance of this certificate was coordinated through the NIST Standard Reference Materials Program by J.C. Colbert.

Willie E. May, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Revised Certificate Issue Date: 5 May 1999
See Certificate Revision History on Last Page

Thomas E. Gills, Chief
Standard Reference Materials Program

Coordination of the measurements leading to certification of this SRM and its update was performed under the direction of J. Brown Thomas, K.E. Sharpless, and M.J. Welch of the NIST Analytical Chemistry Division. Preparation of this SRM and the analytical determinations for its certification and updates were performed by J. Brown Thomas, R.G. Christensen, P.M. Ellerbe, L.T. Sniegoski, and E. White V of the NIST Analytical Chemistry Division. Analytical determinations of fat and fatty acids were performed by Covance Laboratories (Madison, WI), Lancaster Laboratories (Lancaster, PA), Medallion Laboratories (Minneapolis, MN), and Southern Testing and Research Laboratories (Wilson, NC).

Consultation on the statistical design of the original experimental work was provided by R.C. Paule of NIST. Statistical consultation for the updates was provided by L.M. Gill and S.B. Schiller of the NIST Statistical Engineering Division.

NOTICE AND WARNING TO USERS

Storage: Sealed ampoules should be stored, as received, in a refrigerator at a temperature between 2 °C and 8 °C. The ampoules should not be exposed to light.

Use: Prior to use, samples should be thawed by submerging each ampoule in warm water (25 °C to 30 °C) and sonicating for approximately 5 min to 10 min. Samples for analysis should be withdrawn immediately after opening ampoules and should be processed without delay for the certified values in Table 1 to be valid within the stated uncertainties. Certified values are not valid for material stored in previously opened and resealed ampoules.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

SOURCE AND PREPARATION OF MATERIAL

The natural coconut oil for this SRM was supplied by ICN Nutritional Biochemicals, Cleveland, OH. Approximately 500 mg/kg of 2,6-di-*t*-butyl-4-methylphenol (butylated hydroxytoluene; BHT) were added to prevent oxidation. The oil was homogenized by stirring for 24 h in a heated 25-L glass container before being subdivided into two 11-L batches. To prepare the fortified oil, SRM 911a Cholesterol and vitamins obtained from commercial sources were added to one of the batches of natural oil. These compounds were dissolved in HPLC-grade toluene prior to spiking the oil. The gravimetric concentrations were corrected for purity. The purities of retinyl acetate and *dl*-α-tocopheryl acetate were determined by comparing the absorbances of dilute solutions with accepted literature values for the absorptivities of the compounds. Because certain impurities absorb at the wavelengths at which the absorbance was measured, potential errors from these interferences were compensated for by performing liquid chromatographic (LC) analysis, by which the ratio of the absorbance due to the substance of interest was determined. The purity of the ergocalciferol was determined by combining results from this method with results from differential scanning calorimetry (DSC).

ANALYSIS

Determination of Retinyl Acetate and Ergocalciferol: Aliquots of the oil were originally analyzed for retinyl acetate and ergocalciferol by on-line multidimensional normal-phase LC [2]. The procedure uses gel permeation chromatography (GPC) on a 10-μm gel column (50 Å pore size) to eliminate the bulk of the lipid material in the coconut oil matrix prior to separation of the vitamins using normal-phase LC on a polar, chemically bonded, semi-preparative aminocyano column. The coconut oil was diluted with an internal standard solution (tocol in hexane) and injected onto the gel column. After elution of the lipid material, the valve was switched, and the vitamins eluting from the gel column were introduced onto the aminocyano column. Ultraviolet (UV) absorbance detection at 292 nm was used. Mass fractions of retinyl acetate and ergocalciferol were determined from peak area ratios of the analyte and internal standard. Measurements for the reanalysis of these constituents were performed using the multidimensional normal-phase LC procedure in an off-line mode.

Determination of *dl*-α-Tocopheryl Acetate: An aliquot of the oil was diluted with an internal standard solution of tocol in hexane and fractionated on the semi-preparative aminocyano column, using the same chromatographic conditions as described previously, to obtain a fraction containing *dl*-α-tocopheryl acetate and tocol. The fraction was evaporated to dryness under a nitrogen stream and reconstituted with HPLC-grade acetonitrile for reversed-phase LC analysis on a polymeric C_{18} column with UV absorbance detection at 284 nm.

Determination of Total Cholesterol: Total cholesterol was determined by modification of the combined ID/GC/MS method for serum [3,4]. A known amount of isotopically labeled cholesterol was added to an oil sample; the sample was subjected to hydrolysis under conditions similar to those of the Association of Official Analytical Chemists (AOAC) method for the determination of cholesterol in vegetable fats and oils [5]; and the mixture of labeled and unlabeled cholesterol was isolated and converted into trimethylsilyl ethers. Intensity ratios for selected ions representative of the labeled and unlabeled cholesterol in samples, bracketed in both time and ratio by standards of known composition, were measured by GC/MS. The mass fraction of cholesterol was calculated from the quantity of oil, the quantity of labeled cholesterol added, and the measured intensity ratios for samples and standards.

Determination of Fat and Fatty Acids: Four collaborating laboratories (Covance, Lancaster, Medallion, and Southern Testing and Research) each measured fatty acids in three ampoules each of SRM 1563-1 (natural) and 1563-2 (fortified) coconut oil. Samples were hydrolyzed and analyzed by GC. Fat was reported as the sum of the fatty acids. Assigned values and associated uncertainties were calculated from the equally weighted means of results reported by the four laboratories.

Table I. Certified Concentration Values for Cholesterol and Vitamins in SRM 1563-2 (Fortified Oil)

Constituent	Mass Fraction (mg/kg)	Concentration (mg/L)[a]
Cholesterol[b,c]	638 ± 8	585 ± 6
Ergocalciferol[c,d]	10.9 ± 0.8	10.0 ± 0.7
dl-α-Tocopheryl Acetate[c,e]	158 ± 6	145 ± 6

[a] These values are provided for user convenience and apply only at 23 °C (density of the oil is 0.918 g/mL ± 0.0009 g/mL), but should change only slightly between 19 °C and 27 °C.

[b] The certified value and associated uncertainty for cholesterol in the fortified oil, 1563-2, are based on a straight line modeling of the degradation in cholesterol as a function of time from 1987 through 2001. A two-sided 90 % confidence interval was placed around this line. The interval defined by the certified value and uncertainty extends from the lower bound for the predicted concentration of cholesterol in 2001 to the upper bound for cholesterol in 1987. At any point in time through 2001, this interval will cover the true concentration with a confidence level of 95 %.

[c] The certified values and associated uncertainties were derived from the weighted combination of (a) ID/GC/MS and gravimetric data for cholesterol and (b) LC and gravimetric data for the vitamins. This procedure is described elsewhere [6]. The uncertainties for ergocalciferol and *dl*-α-tocopheryl acetate are two standard deviations of the certified values and include both within and between analytical method differences.

[d] The literature value for the absorptivity of ergocalciferol is 458.9 dL/g·cm at 265 nm in hexane [7].

[e] The literature value for the absorptivity of *dl*-α-tocopheryl acetate is 43.6 dL/g·cm at 285 nm in ethanol [8].

Table 2. Reference Concentration Values of Fat and Fatty Acids (as Triglycerides)[a]

NOTE: These concentrations are provided as reference values because the results have not been confirmed by an independent analytical technique as required for certification.

Constituent	SRM 1563-1 (Natural Oil) Mass Fraction (%)	SRM 1563-2 (Fortified Oil) Mass Fraction (%)
Fat	99 ± 3	97 ± 4
Decanoic Acid (C10:0) (Capric Acid)	5.8 ± 0.6	5.8 ± 0.3
Dodecanoic Acid (C12:0) (Lauric Acid)	46 ± 1	45 ± 2
Tetradecanoic Acid (C14:0) (Myristic Acid)	18 ± 1	18 ± 1
Hexadecanoic Acid (C16:0) (Palmitic Acid)	9.4 ± 0.9	9.2 ± 0.9
Octadecanoic (Acid) (C18:0) (Stearic Acid)	2.8 ± 0.4	2.7 ± 0.4
9-Octadecenoic (C18:1) (Oleic Acid)	7 ± 1	6.6 ± 0.9

[a] Each reference concentration value, expressed as a mass fraction, is an equally weighted mean of results reported by the collaborating laboratories. The uncertainty in the reference values is expressed as an expanded uncertainty, U, at the 95 % level of confidence and is calculated according to the method described in the *ISO Guide to the Expression of Uncertainty in Measurement* [9]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Because the fortified oil was prepared by adding vitamins dissolved in toluene, the fortified oil may contain slightly lower levels of the naturally occurring fatty acids than does the natural oil, although the difference is probably not statistically significant.

Table 3. Information Concentration Values for Cholesterol and Vitamins

NOTE: These concentrations are provided as information values only because of the low levels of the constituents (in the case of 1563-1) or because the concentration has changed since the original certification (in the case of 1563-2) and data are not available to determine the rate of degradation. The data for these information values are not of sufficient quality or quantity to adequately assign uncertainties.

	Constituent	Mass Fraction (mg/kg)	Concentration (mg/L)[a]
1563-1 (Natural Oil)			
	Cholesterol	3.4	3.1
	Retinyl Acetate	<1	<0.9
	Ergocalciferol	<1	<0.9
	dl-α-Tocopheryl Acetate	<1	<0.9
1563-2 (Fortified Oil)			
	Retinyl Acetate	8.2[b,c]	7.5

[a] These values are provided for user convenience and apply only at 23 °C (density of the oil is 0.918 g/mL ± 0.0009 g/mL) but should change only slightly between 19 °C and 27 °C.

[b] The value for retinyl acetate has changed since the original certification, with measurements on four ampoules ranging from 8.1 mg/kg to 8.6 mg/kg.

[c] The literature value for the absorptivity of retinyl acetate is 1520 dL/g·cm at (327 to 328) nm in cyclohexane [10].

Table 4. Information Concentration Values of Fatty Acids (as Triglycerides)[a]

NOTE: These concentrations are provided as information values only because the disagreement among the results was greater than expected for reference values or because results were reported by a limited number of laboratories. The data for these information values are not of sufficient quality or quantity to adequately assign uncertainties.

Constituent	SRM 1563-1 (Natural Oil) Mass Fraction (%)	SRM 1563-2 (Fortified Oil) Mass Fraction (%)
Hexanoic Acid (C6:0) (Caproic Acid)	0.74	0.69
Octanoic Acid (C8:0) (Caprylic Acid)	7.8	7.6
Linoleic Acid (C18:2)	1.6	1.5
Eicosanoic Acid (C20:0) (Arachidic Acid)	0.094	0.10

[a] Each information concentration value, expressed as a mass fraction, is an equally weighted mean of results reported by the collaborating laboratories. Because the fortified oil was prepared by adding vitamins dissolved in toluene, the fortified oil may contain slightly lower levels of the naturally occurring fatty acids than does the natural oil, although the difference is probably not statistically significant.

REFERENCES

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Certificate Revision History: 5 May 99 (This revision reports updated concentration values for cholesterol in the fortified coconut oil and the addition of fat and fatty acid concentrations.) 7 Nov 96 (change in retinyl acetate concentration) 8 Dec 95 (update in cholesterol concentration) 21 Jul 87 (original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: Telephone (301) 975-6776 (select "Certificates"), Fax (301) 926-4751, e-mail srminfo@nist.gov, or via the Internet: http://ts.nist.gov/srm.